THINK INDIA JOURNAL

ISSN:0971-1260
Vol-22- Issue-35-December-2019

Analytical Method And Development For The Determination Of Benzalkonium Chloride In Betaxolol (0.5%) Ophthalmic Formulations

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ABSTRACT

High-performance liquid chromatography has been utilized to quantitative benzalkonium chloride (alkylbenzyldimethylammoniumchioride) in complex ophthalmic details at or beneath fixation levels of 50 ppm. The method includes a one-advance dilution for sample planning and direct injection; along these lines, recuperation as well as transformation issues are nonexistent. The measure is snappy, explicit, reproducible, and straightforward. This method is snappy, explicit, and particularly helpful for medicate item stability contemplates. Likewise, on the grounds that the method recognizes every homologue, it tends to be reached out to routinely decide the homologue proportion for quality control purposes. The developed method is reasonable for the normal analysis of Benzalkonium chloride in Betaxolol (0.5%) ophthalmic arrangements just as for the stability thinks about.

Keywords: Benzalkonium Chloride, Ophthalmic, Betaxolol, HPLC, Alkyl

INTRODUCTION

The assurance of low concentration additive in pharmaceutical plan establishes a difficult issue in current pharmaceutical analysis. This investigation was directed to develop and approve stability demonstrating High Performance Liquid Chromatography (HPLC) method for assay of benzalonium chloride in betaxolol 0.5% ophthalmic solution

Ophthalmic arrangements are sterile fluid or oily solutions or suspensions of at least one dynamic materials. These items are regularly stuffed in appropriate multi-portion compartments that permit the instillation of progressive drops of the readiness.

Benzalkonium chloride is one of a quaternary ammonium aggravate, a joined blend of alkylbenzyldimethylammonium chlorides containing the chemical equation $[C_6H_5CH_2N(CH_3)_2R]Cl$, where R is an alkyl group alterable from $C_8H_{17}$to $C_{18}H_{37}$. It is a blend of alkylbenzyldimethylammonium chlorides of different even numbered alkyl chain lengths. This item is a nitrogenous cationic surface-acting agent having a place with the quaternary ammonium group. It has three primary classes of utilization; as a biocide, a cationic surfactant and stage move agent in the chemical industry. With a property of antimicrobial agent, benzalkonium chloride can be securely utilized in

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pharmaceuticals at lower concentrations from 0.002% to 0.02% yet it tends to be shift up to 0.2% in ophthalmic details relying upon different elements.

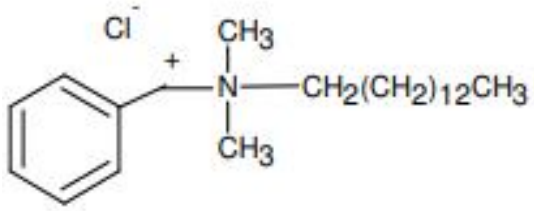

Figure 1: Structure of benzalkonium chloride (BKC)

Betaxolol hydrochloride is a white, crystalline powder, soluble in water, with an atomic load of 343.89. Betaxolol chloride in ophthalmic arrangements has been demonstrated to be successful in bringing down intraocular pressure and is shown in the treatment of ocular hypertension and chronic open-point glaucoma. The chemical structure is available in Figure 2.

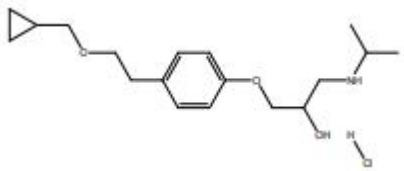

Figure 2: Structural formulas of Betaxolol hydrochloride

As utilization of the proposed method, it was utilized for BKC estimation in different advertised ophthalmic solutions and during being used stability study.

In this work we present a straightforward robust, accurate and precise method for the assurance of benzalkonium chloride in betaxolol (0.5%) ophthalmic solutions.

LITERATURE REVIEW

J. Mehta, K. Patidar and N. Vyas (2010) The analyte was chromatographed on a Waters Spherisorb CN, (4.6x250 mm) section stuffed with particles of 5 µm. The mobile phase, advanced through a trial configuration, was a 40:60 (v/v) blend of potassium dihydrogen orthophosphate cradle (pH 5.5) and acetonitrile, pumped at a flow rate of 1.0 mL/min at keeping up section temperature at 30 ºC. Greatest UV identification was accomplished at 210 nm. The method was approved as far as linearity, repeatability, middle of the road exactness and method precision. The method was demonstrated to be robust, opposing to little deliberate changes in pH, flow rate and structure (organic ratio) of the mobile phase. The method was effectively applied for the assurance of BKC in a pharmaceutical definition of latanoprost ophthalmic solution with no impedance from basic excipients and drug substance.

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Ankit Agarwal, Sunil Tiwari and KashyapNagariya (2013)To develop a basic, fast and accurate HPLC method for concurrent quantitative assurance of Latanoprost, Timolol and Benzalkonium chloride (BAK) in ophthalmic solution. Chromatographic separation was accomplished with PDA indicator utilizing Inertsil C18, 300 x 3.9mm, 5âµ turn around phase systematic section. The mobile phase comprise of cradle: acetonitrile (40: 60 v/v), was gone through the section at flow rate of 1.0 ml/min. The method was performed at wavelength angle .The test was completed at 30oC. The calibration bends were straight in the concentration scope of 25% to 150% of the working concentration (r2 > 0.999). The lower furthest reaches of quantification was 0.8, 0.9 and 0.6 for Timolol, BAK and Latanoprost individually.

Labranche LP, Dumont SN, Levesque S and Carrier A. (2007) A basic and quick turned around phase HPLC method was developed for routine analysis of all out benzalkonium chloride in ophthalmic plans. The analysis includes basic sample preparation utilizing the mobile phase as the diluent. The method utilizes a Waters SymmetryShield RP-18 (75 mm x 4.6 mm, 3.5 microm molecule size) section and a mobile phase comprising of a blend of methanol-potassium phosphate (pH 3.0; 7.5 mM) (68:32, v/v). Utilizing these conditions, three significant homologs of the benzalkonium chloride (C(12), C(14) and C(16)) were separated in less then 7 min. Moreover, recuperations running from 97% to 99% at three degrees of the name guarantee of complete benzalkonium chloride content were acquired for various ophthalmic details. Information supporting the development and approval of this method are displayed.

RakshitKanubhai Trivedi, Swetha Challa ,Mukesh C. Patelb , Dipika R. Trivedi and Parimal M. Chatrabhuji (2013) A stability-demonstrating RP-UPLC method was developed for the concurrent assurance of fluticasone furoate (FF) and benzalkonium chloride (BKC) in a pneumonic drug item. The ideal chromatographic separation was accomplished on the BEH C18, 1.7 µm (50x2.1 mm) segment, utilizing isocratic elution at 215 nm finder wavelength. The improved mobile phase comprised of 0.05 M potassium dihydrogen phosphate cushion and acetonitrile in the ratio of 45:55% v/v. The developed method separated FF and BKC inside 5 minutes. The stability-demonstrating capacity was set up by constrained debasement tests. Moreover, this method can be stretched out for singular estimation of FF and BKC in different economically accessible aspiratory drug items.

BhaskarDaravath, GouruSanthosh Reddy and KamarapuSK(2014) The present investigation depicts method development and ensuing approval of RP-HPLC method for the synchronous estimation of Diethylcarbamazine citrate and Chlorpheniramine maleate in tablet dosage structures. Switched phase high-performance liquid chromatography (RP-HPLC) method was developed and approved for synchronous estimation of Diethylcarbamazine citrate and Chlorpheniramine maleate in consolidated dosage structures. RP-HPLC separation was accomplished by utilizing

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Kromasil C18 segment (250mm 4.6mm, 5mm) with mobile phase comprising of (80:20) Acetonitrile: Potassium di hydrogen phosphate solution (0.01M, pH 3.0 modifying with Ortho phosphoric corrosive) with a flow rate 1.0 ml/min(UV location 238nm).

MATERIALS AND METHODS

Chemicals

Benzalkonium chloride working standard (100% immaculateness) got from Merck, Germany. Acetonitrile and frosty acidic corrosive (HPLC Grade, SDFCL). Sodium acetic acid derivation, disodium edetate sodium chloride (CharloErba) Purified water is. Ltd. Betxalol standard (99% virtue) and betaxolol 0.5% ophthalmic solution samples.Gotten from Bash Pharma, Co Sudan.

Instruments

Analysis was performed on High Performance Liquid Chromatography-HPLC (SHIMADZU, JAPAN) outfitted with UFLC line pump (model LC-20AB) and Prominence auto sampler (model SIL-20AC), Column L10, CN (250 mm × 4.6, 10 µm). Noticeable quality UV/VIS Detector (model SPD-20AV), Prominence Degassing Unit (mode DGU20A 3 R) and section broiler (model CTO-20A). Mettler Toledo Balance MS model 1050 DU (Switzerland). Ultrasonicater (Model Elmasonic S80, Germany). Nylon Filter 0.45 µm. Information securing was made with SHIMADZU LC-Solution programming.

Liquid chromatographic conditions

Injection volume 100 µL, flow rate; 2.0 mL/minute, discovery wavelength of 254 nm;column stove 30°C mobile phase (0.1M sodium acetic acid derivation, acetonitrile (55:45 v /v).

Methods

1. **Mobile phase preparation:**Precisely 0.1M Sodium acetic acid derivation buffer powder was readied and the pH was changed in accordance with 5.00 with icy acidic Acid. The mobile phase was set up by mixing 0.1 M sodium acetic acid derivation buffer and acetonitrile (45% 55%, v/v). The mixture was sifted and degassed for 10 minutes by sonication.
2. **Benzalkonium chloride standard stock solution:**Precisely 100 mg of benzalkonium chloride standard were accurately gauged and moved into a 100 ml volumetric flask; the volume was finished up to check utilizing purged water and sonicated for 1 moment to create a solution having a concentration of 1000 µg/ml.
3. **Preparation of placebo solution:** The solution containing betaxolol

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excipients (disodium edetate sodium chloride) barring benzalkonium chloride is utilized as fake treatment.

RESULTS AND DISCUSSION

System suitability test

A stock solution of benzalkonium chloride of a concentration of 200 µg/ml was arranged and was injected multiple times and the results got are appeared in Table 1 and the acquired chromatogram is appeared in Figure 3.

Table 1: Results of System suitability test.

Injection #	Ret. Time		Sum of Peak Area	Theo. Plate		Tailing Factor	
	C12	C14		C12	C14	C12	C14
1	8.2	10.3	209856	3555	3686	1.058	0.954
2	8.2	10.3	209775	3629	3859	1.058	0.954
3	8.2	10.3	210369	3620	3882	1.057	0.951
4	8.2	10.2	209576	3624	3896	1.058	0.96
5	8.1	10.2	209485	3628	3878	1.057	0.95
Average	8.18	10.26	209812.2	3611	3840	1.058	0.954
STDEV	0.04	0.06	345	31.62	87.21	0.001	0.004
RSD%	0.55	0.53	0.16	0.88	2.3	0.05	0.41

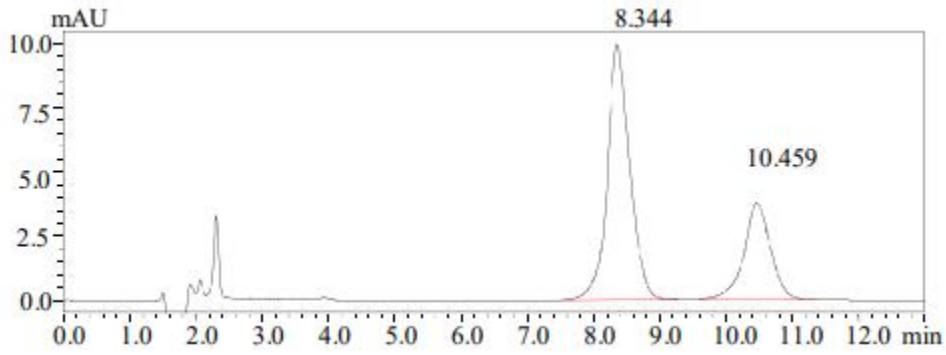

Figure 3: The chromatogram of benzalkonium chloride

Stability of solution

A benzalkonium chloride solution (200ug/ml) was injected into HPLC framework as fresh sample. and afterward was injected following 6 hours and following 24 hours. A fresh test solution was arranged and investigated following six hours and 24 hours. At each time, the sample was investigated multiple times. Interady RSD% were 0.19, 33 and 33%. RSD% of entirety tops region of fresh injected working standard of benzalkonium chloride, following 6 hours and following 24 hours was determined and saw as 0.28%.

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Linearity

A progression of seven concentrating levels (100.15-160.24, 180.27, 200.30, 220.33, 240.36 and 260.39 µg/ml were set up from the stock solution (1000 µg/ml) and the solutions were estimated and the calibration bend was plotted. The relapse condition acquired was: Y=1051.6x - 8405.4, R^2 =0.9995.

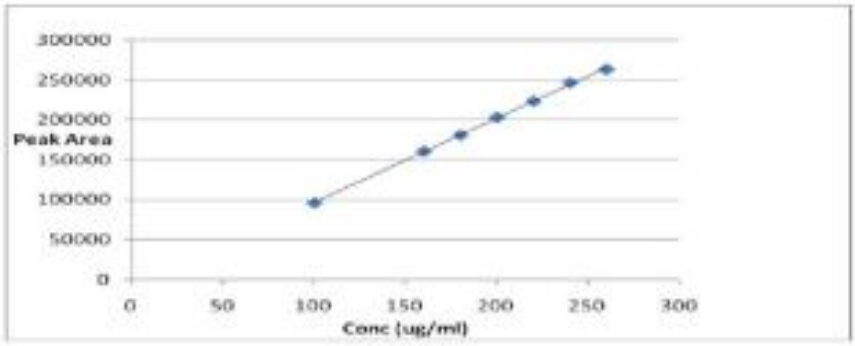

Figure 4: The Calibration Curve plot of benzalkonium chloride

Specificity

Placebo of the betaxolol (0.5%) ophthalmic solution, equal to the sample volume was taken and solution arranged and investigated. No interferents peaks were appeared in the got chromatogram.

Accuracy

Three unique quantities (low, medium and high for example 80%, 100% and 130% of the standard test solution) of the legitimate standard were arranged and injected in triplicate for each spike level. The results acquired were adequate (acknowledgment recuperation criteria % is 98%-102 %). See Table 2.

Table 2: Results of the recovery study

Conc.	80%	100%	130%
Avg. assay.(n=3)	79.95%	100.23%	129.61%
Avg. recovery	99.94%	100.23%	99.70%
RSD%	1.57	1.56	0.73

Precision

Investigation of the precision of the assay was controlled by repeatability (intra-day) and middle precision (between day) in triplicate. Repeatability was assessed by assaying of eight judgments at 100% of the test concentrations. Moderate precision was surveyed by looking at the assay of eight judgments at 100% of the test concentrations on various days (3 days) arranged in a similar way for repeatability.

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The RSD% acquired was

1. **Intra-day precision:**

Table 3: Benzalkonium chloride results of the intra-day precision test (repeatability)

	AverageAssay (n=3)	STDEV	RSD%
Day 1	99.95	0.398	0.40
Day 2	100.39%	0.328	0.33
Day 3	101.96%	0.594	0.58

2. **Intermediate precision:**The general intermediate precision of the method is appeared in Table 4. The results were inside the satisfactory range, for example RSD ≤ 2.

Table 4: Overall intermediate precision results

Days	Avg. Assay	STDEV	RSD%
Day 1	99.95	1.056	1.05
Day 2	100.39		
Day 3	101.96		
Overall average	100.77		

Robustness

The Robustness was dictated by injecting triplicate injections of standard solution. The parameters tried were, flow rate, section broiler temperature and recognition wavelength mobile phase pH.

CONCLUSION

This investigation displays a basic approved HPLC method for estimation of BAC in an assortment of ophthalmic preparations. The developed method is explicit, quick, robust, precise and accurate. A basic, sensitive, cost-viable method for the assurance of benzalkonium chloride in betaxolol 0.5% ophthalmic solution was developed and approved. The developed method meets the ICH rules for method approval.

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